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Alpha Emitting Nuclides for Targeted Therapy

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Abstract

Targeted alpha therapy (TAT) is an area of research with rapidly increasing importance as the emitted alpha particle has a significant effect on inducing cytotoxic effects on tumor cells while mitigating dose to normal tissues. Two significant isotopes of interest within the area of TAT are thorium-227 and actinium-225 due to their nuclear characteristics. Both isotopes have physical half-lives suitable for coordination with larger biomolecules, and additionally actinium-225 has potential to serve as an *in vivo* generator. In this review, the authors will discuss the production, purification, labeling reactions, and biological studies of actinium-225 and thorium-227 complexes and clinical studies.

Keywords

Thorium-227; Actinium-225; alpha therapy; radiolabeling

Introduction

Targeted Alpha Therapy (TAT) is a growing area of investigation in the field of radiopharmaceuticals. A review performed using the Web of Knowledge, showed that since the first treatment of a leukemia patient with an alpha emitter in 1995[1], publications in this area have increased approximately 1500%. There have been a few reviews and editorials that examine different aspects of TAT[2–8]. This review will present an update on actinium-225 (^{225}Ac) and thorium-227 (^{227}Th) chemistry, radiochemistry, chelators, production of isotopes and preclinical and clinical examples.

Pre-clinical and clinical investigations of alpha particle emitters have been driven in part by increased isotope availability, understanding and development of safe handling protocols, and development over the years of libraries of bifunctional chelators for conjugation of radionuclides to targeting vectors. The isotopes discussed herein show promise for TAT as they both emit multiple alpha particles in their decay chains.

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The moderate pathlength (50 – 100 μm) and high linear energy transfer (LET: 80 keV/μm) of alpha particles appear to be suitable for disseminated disease, small neoplasms, micrometastases and elimination of single tumor cells. Thus, alpha particles have been considered complementary to the long pathlength (<12 mm) and low LET (~ 0.2 keV/μm) of beta particles that are effective in large, heterogeneous tumors where the long β-range evenly distributes the radiation dose. The long range of beta particles can also result in irradiation of healthy tissue surrounding the tumor site. Recent clinical studies show that alpha emitters may be effective in controlling bulky disease[9] and in overcoming treatment resistance to beta particle therapy

An alpha particle is a $^{4}He^{+2}$ nucleus with an immense mass compared to electrons and is monoenergetic with initial kinetic energy between 5 and 9 MeV. Unlike beta particles, alpha particles maintain an almost linear path with destruction occurring all along the path and not just primarily at the end of the path. Interaction of alpha particles with biological tissue launches complex molecular pathways[6]. The primary target of the alpha particle's high LET radiation is DNA, where double strand breaks result from a single alpha particle track resulting in cell sterilization[10]. In contrast, beta particle irradiation produces mainly ionization resulting in single strand breaks, and exhibits about 500 times lower cytotoxicity[6].

Secondary effects may enhance the ability of alpha and beta particles for cell killing. The ability of a particle to damage neighboring cells is called the "crossfire effect". The cross-fire effect is operative with beta emitters due to their longer range in tissue and is advantageous in heterogeneous tumors, Figure 1. However, recent clinical studies demonstrate that alpha particles possess noteworthy therapeutic effects on large tumors and large bulky disease although this may be due to other mechanisms[9, 11–12]. To wit, alpha emitters have been applied using DOTATOC[13] for neuroendocrine tumors and a prostate-specific membrane antigen (PSMA) ligand for prostate cancer[14]. The short range of alpha particles in tissue may result in an irradiation that is truly targeted to tumor cells with minimal effects on normal tissues.

Another mechanism operative in enhancing therapeutic effects is the "radiation-induced bystander effect" (RIBE), Figure 1. This effect results in cells that are in the proximity of cells exposed to ionizing radiation but themselves not directly exposed, behaving as if they have been exposed[9]. While the exact mechanism of RIBE is not known, it is likely that information is transmitted from irradiated cells to neighboring cells by stress signal factors. Finally, the "abscopal effect" is a phenomenon that has been observed in a variety of tumors and is attributed to irradiation-induced immune mechanisms that result in a therapeutic response in remote lesions.[15] The cell-killing efficiency of alpha particles is independent of cellular oxygenation.[16]

For optimized therapeutic efficacy, the α-cytotoxic payload should accumulate selectively in diseased tissue and deliver a suitable radiation dose to tumor sites while sparing normal organs and surrounding healthy tissue. In order to achieve optimized therapeutic efficacy, it is important to match the physical half-life of the alpha particle, and relative biological effectiveness to tumor mass, size, and biological half-life of the vector. Unlike beta particles, alpha particles have high linear energy transfer (LET) with a mean energy deposition of

100 keV/μm. This can result in DNA double strand breaks without the use of oxygen, unlike beta particles, and thus are effective in hypoxic tissues. Cell death can result from only a single alpha particle, and can be dose dependent[17]. Additional advantages of alpha particles are severe chromosome damage even at mitosis, destruction of cells independent of cell cycle, and as demonstrated in the clinic, the potential to overcome resistance. While radium dichloride exhibits bone-targeting properties due to its similarity to calcium (*vide infra*), most alpha emitters require conjugation to targeting vectors to deliver the α-cytotoxic payload to cells expressing high target concentrations[1–2]. Certainly, ^{225}Ac and ^{227}Th, as members of the actinide series, require chelators for stabilization and attachment to targeting molecules. Targeting molecules are generally antibodies, peptides and small molecules. Antibodies are exquisitely selective for their antigens expressed on tumor cells resulting in high tumor uptake and low accumulation in healthy tissue. However, antibodies possess long blood circulation time (generally days) that increases the risk of hematotoxicity and myelotoxicity. Alternatively, small molecules and peptides can exhibit high tumor penetration and faster clearance than antibodies[9].

While TAT is a growing area of interest and research, there is currently only one alpha emitting radioisotope which has been approved for treatment by the food and drug administration. Radium dichloride ($^{223}RaCl_2$) marketed under the name Xofigo, has been approved for the palliative treatment of castration resistant metastatic prostate cancer tumors in bone[18]. In this case, no bifunctional ligand nor targeting vector is required. Radium (Ra^{2+}) is a calcium mimic, and as a metal ion binds to areas of increased bone turnover in skeletal metastases[19]; subsequently double strand DNA breaks induce apoptotic effects[20]. Here, the short range and high LET of the emitted alpha particles result in optimized targeting and mitigate adverse effects to surrounding normal tissue[21–22].

Handling Alpha Emitting Radionuclides

Handling alpha emitting radionuclides is an important issue with the main concern being internalization and deposition in healthy tissues. Due to the short range of alpha emitters, they can be shielded by gloves, as they do not constitute an external radiation hazard. In handling of alpha emitters researchers need to consider the specific radionuclide as well as its progeny. While the Geiger-Muller survey meter is the usual probe in a radiochemistry lab, in the case of alpha emitters, researchers should additionally employ an alpha probe consisting of a ZnS(Ag) scintillator and a photo-multiplier (PM) tube [23]. All meters should be properly calibrated and run at the optimal operating voltage of the PM tube for the specific radionuclide. Allowable removable contamination levels for alpha emitters is 50 times less than for beta emitters. Thus, it is critical to mitigate contamination as much as possible to achieve low levels of contamination to be compliant with regulations. In addition, alpha probes are generally less efficient than other detectors thus making the detection of alpha contamination somewhat challenging. Researchers should work with alpha emitters that possess low abundance and low energy gamma emission in a well-ventilated hood or glove box. Alternatively, handling alpha emitters that emit high energy γ-rays, should be performed in a hot cell or behind 6-inch lead bricks using remote handling techniques. Double gloving should be employed, and wipe tests should be performed with analysis using a gamma counter and/or liquid scintillation counter.

Actinium-225

General Chemistry and Radiochemistry

Actinium is a radioactive element with atomic number 89 and possesses 32 isotopes. Of the 32 isotopes, ^{227}Ac and ^{228}Ac are present in nature as a result of the naturally occurring ^{235}U and ^{232}Th decay chains. The chemistry of actinium is poorly developed owing to its limited supply and special facilities and handling required of all Ac isotopes.

Actinium usually exists as a +3 ion in aqueous solution and is considered to possess chemical properties similar to lanthanum +3. In fact, La^{3+} is often used as a nonradioactive surrogate for Ac^{3+}. The 6-coordinate ionic radius of La^{3+} (1.03 Å) is smaller than Ac^{3+} (1.12 Å)[24]. The low charge density renders Ac^{3+} a very basic +3 ion. The first hydrolysis constant, pK_{1h}, represents the ability of the metal to polarize coordinated water to favor release of a proton and formation of $AcOH^{2+}$, and for Ac^{3+} this was measured by an ion exchange method and determined to be 9.4 ± 0.1[25]. This study also measured pK_{1h} of La(III) as 9.0 + 0.1 under similar conditions. Other studies show the first hydrolysis constant of La^{3+} to be 8.63 by different methods[26]. These studies suggest that the first hydrolysis constants are consistent with the charge densities of Ac^{3+} and La^{3+}. This also suggests that Ac^{3+} is a “hard” metal ion. Practically speaking, this information suggests that basic conditions may be used for radiolabeling of Ac complexes.

Spectroscopically Ac^{3+} is invisible to many forms of routine spectroscopy, such as ultraviolet-visible, fluorescence, electron paramagnetic resonance, etc. due to its electronic configuration ($5f^0$ $6d^0$). Using the long-lived isotope ^{227}Ac ($t_{1/2}$: 21.772 y) Ferrier *et. al.* measured the L3-edge X-ray absorption near-edge structure (XANES) representing the first actinium XANES measurement. This study bodes well for study of Actinium via X-ray absorption spectroscopy (XAS)[27]. The interpretation of the extended X-ray absorption fine-structure (EXAFS) data from room temperature solutions containing Ac in HCl demonstrated that the Ac^{3+} was coordinated to ~ 3 Cl^- and ~ 6 H_2O inner -sphere ligands. The calculated coordination numbers agreed with experimental values, and this study demonstrated that Ac tends to possess more Cl^- inner sphere ligands than americium consistent with the notion that Ac^{3+} is substantially less polarizing than the rest of the f-elements and confirming it as a hard acid. Later, the group reported a XAFS study wherein 10.9 + 0.5 water molecules were directly coordinated to the Ac^{3+} cation with an Ac-O_{H2O} distance of 2.63 (1)Å[28]. This was in agreement with Molecular Dynamics-Density Functional Theory (MD-DFT) results. Having 11 inner sphere water molecules is reasonable for the large Ac^{3+} ion; this is consistent with coordination numbers determined by EXAFS for other +3 actinide and lanthanide aquo ions. The coordination number of 11 is consistent with the current ligands, discussed below, containing up to 12 donor atoms[2]. The long Ac-H_2O distance is consistent with actinium as the largest +3 cation known.

^{225}Ac decays via alpha emission (6 MeV) with a half-life of 9.92 d to six consecutive daughter isotopes to stable ^{209}Bi (Figure 2). These isotopes include francium-221 (^{221}Fr; $t_{1/2}$= 4.9 min, α decay (100%)), astatine-217 (^{217}At; $t_{1/2}$= 32.2 ms, α decay (99.99%)), bismuth-213 (^{213}Bi; $t_{1/2}$= 60.5 min, α decay (35.94%), β^- decay (64.06%)), thallium-209 (^{209}Tl; $t_{1/2}$= 2.162 min, β^- decay (100%)), lead-209 (^{209}Pb; $t_{1/2}$= 3.23 h, β^- decay (100%)),

and stable bismuth-209 (^{209}Bi)[29]. Since ^{225}Ac itself cannot be detected directly with gamma spectroscopy, as it does not emit a detectable gamma ray, time must be allowed for the detectable daughter, ^{213}Bi to grow in and be observed by gamma detection.

Production of Actinium-225

One method of production for ^{225}Ac is the separation from thorium-229 (^{229}Th). The ^{229}Th is obtained from the decay of uranium-233 typically from waste streams. As reported by Boll *et. al.* [30], to separate the actinium from ^{229}Th, the stock solution is loaded onto a MP1 anion exchange resin in the chloride form preconditioned with 8 M nitric acid (HNO_3) to remove the bulk thorium. The resulting solution containing radium and actinium is evaporated, reconstituted with 8 M HNO_3, and subsequently passed through a second anion exchange column. The eluate is then evaporated and reconstituted in 10 M HCl which is then passed through a third anion exchange column to remove iron (Fe) as the +3 cation. The resulting solution containing radium and actinium is evaporated and reconstituted with 0.1 M HNO_3 and loaded onto an AG50-X4 cation exchange column. The radium is eluted with 1.2 M HNO_3, and followed by the actinium which is eluted with 8 M HNO_3. The final purification step includes evaporation and reconstitution of the actinium with 0.1 M HNO_3, loading onto a second cation exchange column, rinsing the column to remove residual radium, then eluting the final actinium product with 8 M HNO_3. The purity of the final product is 99.6 ± 0.7% with the impurity contributions coming from radium isotopes (^{225}Ra: 0 – 1.1%, ^{224}Ra: 0.02 – 0.06%)[30]. This 5-step process takes approximately 60 d and yields around 3.7 GBq of ^{225}Ac with an 80% yield. Unfortunately, while this method of production is providing material for preclinical and clinical trials, it does not produce a sufficient amount of material necessary for all clinical trials[31].

Actinium can also be produced through the proton spallation of thorium-232 (^{232}Th) as shown by Harvey *et. al.*[32–34] which allows for the production of ^{225}Ac without the use of heavily regulated special material such as uranium-233 (^{233}U). Here thorium samples with a volume of 2.95 cm^3 were encapsulated in a copper enclosure with a thickness of 0.127 cm. The samples were irradiated with an 8 GeV proton beam over a 4-day period. The spallation yields several different radioisotopes of interest in additional to actinium to include ^{225}Ra, ^{223}Ra, and ^{227}Th. The major disadvantage to this production method is the coproduction of long-lived ^{227}Ac ($t_{1/2}$= 21.78 y), where the isotope is less than 0.2% of the final product mixture[35]. While the byproduct has no effect on labelling, it does complicate waste handling. This work has been advanced by the Department of Energy Isotope Program through its Tri-lab efforts brought together to advance the production of ^{225}Ac by irradiations of ^{232}Th. The effort involves using the high energy accelerators at Brookhaven National Laboratory (BNL) and Los Alamos National Laboratory (LANL) to irradiate 10–100-gram targets of ^{232}Th which is then shipped to Oak Ridge National Laboratory (ORNL) for subsequent processing and distribution. At this point they have distributed over 9.25 GBq of material. BNL is upgrading hot cell facilities to enable curie level production and LANL is building a new facility near their accelerator to allow for production. Recently a drug master file (DMF) was submitted for this material to the FDA to enable use in clinical trials. This work was undertaken to provide a robust, year-round, quality supply of ^{225}Ac. TRIUMF is pursuing a similar production route using their 400 MeV accelerator and

shipping targets to Canadian Nuclear Laboratories (CNL) for processing. Studies performed by Dadachova *et. al.* in which she evaluated the impact of the ^{227}Ac content from high energy accelerator produced ^{225}Ac showed that the impact of the ^{227}Ac on biodistribution and dose is negligible[32]. However, its long half-life does pose concerns with licensing and waste.

^{225}Ac can also be produced via the ^{226}Ra(p,2n)^{225}Ac nuclear reaction using low energy protons from a medical cyclotron; this is potentially a beneficial route as it appears to eliminate ^{227}Ac. Apostolidis *et. al.* measured the cross-section of the proton induced reaction and reported that the maximum occurs at 16.8 MeV[36]. Simulations show that the production route does however produce actinium-226 (^{226}Ac) where the activity contribution could be up to 11%. The half-life of ^{226}Ac is relatively short, $t_{1/2}$= 29 h, compared to ^{225}Ac which allows for decay during the processing steps and a decrease in the ^{226}Ac:^{225}Ac ratio over time. Challenges in using ^{226}Ra as the target material is the alpha decay to gaseous ^{222}Rn and the need for recycling of the target material due to limited availability. The ^{222}Rn ($t_{1/2}$=3.82 d) is highly radioactive and due to its gaseous nature is difficult to prevent from spreading. Predictions show that in scaled up productions irradiating 1 g ^{226}Ra targets with 20 MeV proton can produce 3.996 GBq of ^{225}Ac per month[36].

Another route that is being considered by a number of groups, including Argonne National Laboratory, is the photonuclear route on ^{226}Ra(γ,n)^{225}Ra→^{225}Ac. A five-day irradiation can result in roughly 37000MBg of ^{225}Ra which then can be milked every three days to give 3700–7400MBq-of ^{225}Ac. This method also gives ^{225}Ac free of ^{227}Ac. Challenge with this route is the need to use and recycle the ^{226}Ra target and complications with the ^{222}Rn daughter as stated above.

Radiolabeling and chelator development

All clinical tests and most preclinical research have been conducted using ^{225}Ac derived from the decay of ^{229}Th and subsequent radiochemical extraction. Initial efforts in the chelation of ^{225}Ac for *in vivo* purposes were implemented to reduce the toxicity of the radiometal to the liver and bone[29]. This was first evaluated by using citrate where Beyer *et. al.* compared the biodistribution of [^{225}Ac]Ac-citrate to the distribution of [^{169}Yb]Yb-citrate and determined that the actinium citrate complex overall had less than optimal whole-body clearance but had fast blood clearance, low bone uptake, and higher liver uptake compared to the ytterbium congener[37]. In this study, the ^{225}Ac was produced by the irradiation U_3O_8 with 650 MeV protons, separation of Ra by $BaSO_4$ precipitation, and isolation of the pure ^{225}Ac by cation exchange chromatography. These studies were subsequently followed up by chelating ^{225}Ac with conventional ligands such as ethylenediaminetetramethylenephosphonic acid (EDTMP), ethylenediaminetetraacetic acid (EDTA), and *N*-[(*R*)-2-amino-3-(4-nitrophenyl)propyl]-*trans*(*S,S*)-cyclohexane-1,2-diamine-*N,N,N',N",N"*-pentacetic acid (CHX-A-DTPA). *In vitro* studies wherein cation exchange columns were used to determine “free” Actinium (bound to the column) and complexed Ac shows that only DOTA (1,4,7,10-tetrazacyclododecane-*N,N',N",N'"*-tetraacetic acid) demonstrates around 1% free Ac, while the other chelators do not form stable complexes (almost all Ac bound to column). Intermediate behavior was exhibited by ^{225}Ac

DOTMP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethylenephosphonic acid) where 78% complex and 22% free actinium was observed[38]. The *in vivo* stability was evaluated in mouse models and tracked with *in vitro* studies. All Ac ligand complexes except DOTA exhibited significant liver uptake, while the Ac-DOTA complex exhibited 3.29+1.05 %ID/gram liver uptake[39]. Nevertheless, DOTMP has been labeled with ^{225}Ac and evaluated by Henriksen *et. al.* to assess the biodistribution of bone-targeting molecules[40]. The biodistribution in BALB/c mice showed that the [^{225}Ac]Ac-DOTMP complexes localized more readily in the bone versus soft tissues.

While DOTA is routinely used as a chelator for radiometals, including actinium, it is not the ideal chelator for ^{225}Ac. The main challenge when complexing radiometals to the DOTA ligand is the heat necessary to deprotonate the nitrogen donor groups and bind the radiometal within the cyclic ring. Heating during the radiolabeling protocol is particularly problematic if the DOTA is conjugated to a biomolecule, such as an antibody, where heat would destroy the tertiary structure and affinity of the molecule. As of now, commercially available bifunctional DOTA ligands, such as p-SCN-Bn-DOTA, are commonly used. McDevitt discovered a “2 step” method for production of ^{225}Ac antibody constructs[38]. In this approach, the p-SCN-Bn-DOTA was radiolabeled with ^{225}Ac in the first step. The second step consists of conjugation of [^{225}Ac] Ac--DOTA to the antibody. This approach suffers from hydrolysis of the SCN moiety resulting in loss of 90% of the actinium because this is conjugated to non-reactive forms of DOTA. A one-step method was reported by Maguire *et. al*[41]. that afforded products with 30-fold higher specific activities than typically achieved with the two-step method. However, this method requires conjugation of the IgG antibody with > 10 DOTA chelators per construct; this procedure may not be feasible with most antibodies and could reduce the affinity of the radioimmunoconjugate.

Poty *et. al.* reported utilizing inverse electron-demand Diels-Alder (IEDDA) reaction for the synthesis of ^{225}Ac immunoconjugates[42]. In this approach, a DOTA-with pendent tetrazine (Tz) is radiolabeled with ^{225}Ac at 37°C for one hour. Subsequently, the [^{225}Ac]Ac-DOTA-Tz is reacted with the antibody conjugated to transcyclooctene (TCO). The Tz reacts rapidly and bioorthogonally with the antibody-TCO thus forming the ^{227}Ac radioimmunoconjugate.

Additionally, even though the loss of Ac from DOTA is not as drastic as the chelators mentioned above, there is some release of Ac over time as demonstrated in *in vivo* and *in vitro* studies. A study by Horrocks demonstrates an inverse relationship of lanthanide DOTA stability constants with respect to ionic radius[43]. EXAFS studies, vide supra, suggest that Ac^{3+} can accommodate high coordination numbers. These suggest that new chelators should be developed for ^{225}Ac perhaps with expanded cavities and possibly higher number of donor atoms to better match the large ionic radius of Ac^{3+}.

The general instability of first-generation actinium chelators and the requirement of heat for complexation of DOTA gave rise to the development of new chelators. A novel chelate, 1,4,7,10,13,16-hexaazacyclohexadecane-*N,N’,N”,N’”,N””,N’””*-hexaacetic acid (HEHA) Figure 3 was developed with the goal to increase the *in vivo* stability of ^{225}Ac complexes. The chelate is an 18 membered macrocyclic ring, consisting of 12 coordination sites which satisfies the actinium coordination sphere. To achieve a radiolabeling yield

greater than 90%, 100 μL of an actinium stock (10 MBq in 0.1 M HNO_3) was mixed for 30 min with 20 μL of the HEHA ligand (1 X 10^{-2}M) at 40 °C while keeping the pH at 5 with 1.0 M ammonium acetate (NH_4OAc). To biologically evaluate the complex the reaction mixture was purified on a Chelex column and diluted with 2-ethanesulfonic acid (MES) buffer. The resulting solution (92.5 kBq) was intravenously injected into normal female BALB/c mice and results showed the macrocyclic chelator labeled with ^{225}Ac had increased stability and lower overall toxicity when compared to non-macrocyclic ligands. To further investigate the HEHA ligand for preclinical use, the ligand was bifunctionalized with a benzyl isothiocyanate group and conjugated to BL-3 monoclonal antibodies[44]. To radiolabel the HEHA-mAbs, an ^{225}Ac stock (1.85 – 7.4 MBq) in 0.15 M NH_4OAc with a pH of 7.0 was reacted for 30 min at 37 °C with the mAb conjugate (0.3 – 0.4 mg) in the same buffer. To assess the stability of the [^{225}Ac]Ac-HEHA-mAb the construct was incubated in fetal bovine serum at 37 °C and aliquots were analyzed via radio-HPLC. This radiolabeling method resulted in a 60–85% yield and the stability of the radioimmunoconjugate in serum showed to be favorable at very early time points however the complex begins to disassociate after 1 hour.

Another 18 member macrocyclic ligand, *N,N'*-bis[(6-carboxy-2-pyridil)methyl]-4,13-diaza-18-crown-6 (H_2macropa), Figure 3 was developed to further stabilize actinium *in vivo*[45]. In this study the ^{225}Ac was produced by spallation of uranium carbide and separated by a mass separator using the Isotope Separator and Accelerator on-line facility at TRIUMF. Here, ^{225}Ac was separated from ^{225}Ra using a DGA column and isolated in 0.05M HNO_3 for radiolabeling experiments. The H_2Macropa ligand (59 μM) was mixed with 26 kBq of ^{225}Ac in 0.15 M NH_4OAc pH 5.5 – 6 and the radiochemical yield was evaluated by ITLC on silica gel plates. Remarkably a radiochemical yield of 98% was achieved within 5 min at room temperature. The ability to complex actinium at room temperature is very promising for the future of radiolabeling biomolecules with actinium. The biodistribution of the ^{225}Ac-macropa complex was evaluated by intravenously injecting 10 – 15 kBq of the complex into normal female B57BL/6 mice (n= 3). The biodistribution of the complex was evaluated at 15 min, 1 hour, and 5 hours, and the results were compared to “free” actinium as [^{225}Ac]Ac$(NO_3)_3$. The results showed that uptake trends for ^{225}Ac-macropa closely resembled that of [^{225}Ac]Ac-DOTA. The majority of the uptake was found in the urine, bladder, and kidneys where by 1-hour post injection 489.11, 6.23 and 0.40 %ID/g were found in the organs respectively Figure 4 (a–c). The authors found no disassociation of the actinium macropa complex, but they did note there was slower clearance of the complex compared to the DOTA complex, most likely due to the positive charge on the complex. Macropa was bifunctionalized and conjugated to a PSMA/albumin targeting molecule, RPS-070. Radiolabeling proceeded in the same manner and the resulting construct was evaluated in LNCaP tumor bearing mice. The biodistribution showed rapid clearance through the kidneys (52 ± 16% ID/g 4 hours post injection) and localization at the tumor site (13 ± 3% ID/g 4 hours post injection) with observable tumor washout over time[45].

Case Studies: Preclinical

Some of the earliest preclinical studies with ^{225}Ac was performed by McDevitt *et. al.* using actinium-225 as an *in vivo* alpha generator coupled to internalizing monoclonal antibodies[46].

Their initial interest was in ^{213}Bi but its short half-life limited its delivery. Solution they pioneered was what they termed the “atomic” generator; a single ^{225}Ac atom conjugated to the delivery construct[46]. They showed *in vitro*, these constructs specifically killed leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at becquerel (picocurie) levels. Injection of single doses of the constructs at kilobecquerel (nanocurie) levels into mice bearing solid prostate carcinoma or disseminated human lymphoma induced tumor regression and prolonged survival, without toxicity, in a substantial fraction of animals[46].

Anti-angiogenic therapy with ^{225}Ac radiolabeled to the monoclonal antibody E4G10 that targets vascular endothelial cadherin was evaluated in a prostatic tumor model[47]. A two-step radiolabeling method was used in which ^{225}Ac was first labeled to Meo-DOTA-NCS and then the radiolabeled conjugate was attached to the antibody as previously reported[38]. The ^{225}Ac-E4G10 was evaluated in mice bearing LNCaP prostate tumors and compared to controls that consisted of 1% human serum albumin, unlabeled E4G10, and Ac-225 labeled IgG. Treatment with 50 nCi of ^{225}Ac-E4G10 was administered on days 3,[48] 5, 7, and 10. Suppressed tumor growth, enhanced tumor cell apoptosis and prolonged animal survival were observed, without gross or histopathological toxicity in normal tissues or their vasculature. Synchronized administration of ^{225}Ac-E4G10 and paclitaxel resulted in enhancement of the anti-tumor response[47]. Construct was further shown to eradicate bone marrow-derived endothelial progenitors.

These promising results were followed on by the evaluation of single wall carbon nanotube (SWCNT) constructs covalently appended with radiometal chelates of DOTA for ^{225}Ac radiolabeling and desferrioxamine B (DFO) for Zr-89 radiolabeling and the tumor neovascular targeting antibody E4G10[49]. The SWCNT platform were chosen as the scaffold as they are nonimmunogenic, can be modified to incorporate multiple diagnostic and therapeutic modalities, rapidly clear the blood and are cleared via the kidneys. Thus, they offered the opportunity to improve the pharmacokinetics observed for antibodies i.e. slow clearance from the blood and the ability to image uptake. These SWNCT([^{225}Ac] Ac-DOTA)EG410 constructs were evaluated in a tumor xenograft model of human adenocarcinoma (LS174T). PET imaging was performed with the SWCNT([^{89}Zr]Zr-DFO)E4G10 which showed rapid blood clearance in less than an hour and specific tumor accumulation, demonstrating the enhanced pharmacokinetics offered by the SWCNT platform compared to the E4G10 antibody construct. Further, it offered enhanced cargo delivery and higher specific activity compared to the antibody alone, showed therapeutic efficacy, good image contrast and specificity for the target as well as being well tolerated with a safe profile. Therapy resulted in significant tumor volume reductions and improved survival rates as demonstrated by Kaplan Meier curves to the controls[49].

It has been previously reported that during the treatment of prostate cancer as DNA damage occurs there is an upregulation of the androgen receptor associated with DNA repair genes[50–51]. McDevitt *et. al.* sought to take advantage of the over expression of the androgen receptor by attempting to elicit cell death through targeting human kallikrein peptidase 2 (hK2) whose expression is directly regulated by the androgen receptor[52]. The hu11B6 monoclonal antibody, developed as a diagnostic for catalytically active hK2, is internalized into the nucleus once bound to active hK2. Therefore, an ^{225}Ac labeled hu11b6 would also

internalize into the nucleus of the tumor cell inducing DNA double strand breaks leading to cell death. The cell death would in turn cause an increased expression of hK2 due to the DNA repair creating a loop of cell death and target expression. The hu11B6 antibody was conjugated with DOTA to prepare it for labeling as previously reported[53] where the SCN-DOTA was radiolabeled with ^{225}Ac (pH≈ 5.4, temp ≈ 56, time ≈ 42 min), prior to conjugation to the antibody (pH≈ 8.7 temp ≈ 36, time ≈ 52 min). It was reported that the radiochemical yield was 3.7 ± 2.1% while the radiochemical purity was 99.3 ± 0.5%, and the specific activity was determined to be 2.92 ± 2.03 kBq/g. Male athymic BALB/c nude mice were implanted with 1 – 5 X 10^6 LNCaP-AR cells subcutaneously injected into the right and left flanks. Mice were divided into two experimental groups and one control group. The first experimental group was treated with [^{225}Ac]Ac-hu11B6, which recognizes hK2 and internalizes inside the cell. The second experimental group was treated with the specific, non-internalizing version of the drug, [^{225}Ac]Ac- hu11B6-H435A, which recognizes hK2 but does not internalize. The control group was treated with non-specific [^{225}Ac]Ac-huIgG_1 antibody, and each group was administered 11.1 kBq of the labeled mAb. The results showed that mice treated with [^{225}Ac]Ac-hu11B6 survived for a median of 108 days after treatment. While mice treated with [^{225}Ac]Ac-hu11B6-H435A survived for a median of 22.5 days, and the control group survived 18 days after treatment. The cell death caused by [^{225}Ac]Ac-hu11B6 resulted in increased hK2 expression which was confirmed by its observed increase of accumulation at the tumor site over time. This confirms the hypothesis of the alpha particle feed-forward oncoaddition mechanism.

^{225}Ac has been incorporated into liposomes and nanoparticles to overcome some challenges in working with ^{225}Ac or any alpha emitter. A major challenge is that the energy of the decay is sufficient to break chemical bonds resulting in the release of the daughter nuclides from the chelator. This release can then cause normal tissue toxicity. In order to circumvent this phenomenon many groups have investigated the use of alternative chelator systems that can efficiently house ^{225}Ac and its daughters. Sofu, *et. al.* evaluated the use of liposomes to encapsulate multiple ^{225}Ac and to retain its daughters at the tumor site thus reducing toxicity to normal tissues[54]. Liposomes had been used previously to selectively deliver radionuclides to tumors and sites of infection for diagnosis[55–56]. The ^{225}Ac was passively trapped in the liposomes and at several time points the liposomes were separated from the mother liquor and measured by gamma spectroscopy to analyze for the gamma emission from ^{213}Bi. Pegylated phophatydylcholine-cholesterol liposomes of various sizes and containing different charges were manufactured and used to trap more than 2 Ac atoms. Zwitterionic liposomes demonstrated the highest retention of upwards of 88% out to 30 days. Cationic liposomes exhibited lower retention. It was theoretically calculated that liposomes sized in excess of 650 nm in diameter were needed to retain greater than 50% of the ^{213}Bi daughters. Experimental results indicated retention was 10% less than results predicted by the theoretical models. Studies indicated liposomes could result in delivery of multiple ^{225}Ac but very large liposomes would be need which could limit the application to locoregional therapies.

Woodward[48] developed 3–5 nm diameter monazite ($LaPO_4$) nanoparticles (NP) as carriers for ^{225}Ac. ^{225}Ac[$LaPO_4$] NP were synthesized and conjugated to one of two antibodies, mAb-201B or mAb-33. The NP only partially retained daughters with more than 50%

of the ^{221}Fr and ^{213}Bi released from the NP lattice. Addition of two layers of $LaPO_4$ reduced leaching of ^{221}Fr to 20% [57]. Robertson *et. al* [58]. investigated the use of multi-shell nanoparticles to encapsulate ^{225}Ac and its daughters. Nanoparticles with 4 $GdPO_4$ shells followed by a gold coating demonstrated greatest retention of ^{225}Ac (>99.99%) and its daughters, with up to 98% of ^{221}Fr retained. Although multilayered NP exhibited higher retention properties, the multistep synthesis is very time consuming. Salvanou *et. al.*[59] examined the use of ^{225}Ac labeled macrocycle Au nano particles in brachytherapy. Gold NP were synthesized, reduced in the presence of TADOTAGA ligands to yield Au-TADOTAGA, then radiolabeled with ^{225}Ac. A retardation of tumor growth was observed with a dose of 0.25 kBq/ml. Cedrowska *et. al.* [60] examined the use of ^{225}Ac labeled titanium dioxide (TiO_2) nanoparticles for TAT (REF). TiO_2 nanoparticles modified with substance P, a peptide fragment that targets NK1 receptors on glioma cells trough the silane-PEG-NHS linker were labeled with ^{225}Ac. The labeled bioconjugates displayed leaching of ^{221}Fr, a first decay daughter of ^{225}Ac, in cerebrospinal fluid after 10 days. ^{225}Ac binds to the nanoparticles via exchange with a hydroxyl group on the surface, it is believed that the recoil energy of the alpha decay is distributed throughout the entire nanoparticle and is therefore reduced. The absorbed recoil energy minimizes the path of the daughters enabling their recapture by TiO_2, a known cation exchanger for +3 ions. Cytotoxicity *in vitro* remained high in T98G glioma cells. The ^{225}Ac labeled TiO_2 reduced metabolic activity of the tumors while delivering smaller does to surrounding tissues.

Cornell prime dots (C' Dots) are next generation ultra-small fluorescent core-shell silica nanoparticles that can be tracked due to dyes encapsulated in the core of the particle, which emit fluorescence in the near -infrared region. One advantage to using these ultra-small nanoparticles (<10 mm) for tumor targeting is their clearance by renal filtration. C' dots were functionalized with the α melanocyte-stimulating hormone (αMSH) which targets the melanocortin-1 receptor (MC1R) and radiolabeled with ^{225}Ac to evaluate the tumor microbiome environments after exposure to the immunotherapeutic. Urbanska *et. al.* reported the biodistribution of DOTA chelated [^{225}Ac]Ac-αMSH-PEG- Cy5-C'in both healthy and tumor bearing C57BL/6J mice, where 11.1 kBq were administered in both studies[61]. In normal mice, high accumulation of the ^{225}Ac nanoparticles was observed in the blood (25.37 ± 8.87 %ID/g, 1-hour post injection, n= 3) and were excreted through the renal track (149.9 ± 96.1 %ID/g, n= 3). Throughout all time points the organs with the highest accumulation of the nanoparticles were the liver (7.02 ± 0.35 %ID/g), spleen (6.58 ± 1.86 %ID/g), and kidney (6.52 ± 0.54 %ID/g). In the B16-F10 tumor bearing mice, there were similar trends at early time points, where significant accumulation was found in the blood (22.47 ± 10.39 %ID/g, 1-hour post injection, n= 5) and clearance through the kidneys (28.07 ± 42.15 %ID/g, 1 hour post injection, n= 5). By 1-hour post injection 5.30 ± 1.71 %ID/g (n= 5) was observed at the tumor site. Despite targeting the tumor, there was significant localization in the liver (4.79 ± 0.36 %ID/g), spleen (3.61 ± 1.38 %ID/g), and kidney (4.62 ± 1.38 %ID/g). A therapeutic study was also performed using C57BL/6J mice subcutaneously implanted with B16-F10 cells which grew 8 to 10 days prior to treatment. Mice were divided into three treatment groups, and administered either: 11.1 kBq of [^{225}Ac]Ac-αMSH-PEG-Cy5-C', 11.1 kBq of the non-targeting [^{225}Ac]Ac-αNH_2-PEG-Cy5-C', or 1% human serum albumin. Mice treated with the targeted drug had a median survival time of 26 days, mice

treated with the non-targeted version had a median survival time of 21 days, and the control group injected with the 1% human serum albumin solution had a median survival time of 14 days. The tumor microenvironment of mice administered [^{225}Ac]Ac-αMSH-PEG-Cy5-C' was evaluated and it was determined that the pharmacological consequence of the emitted alpha particles and the nanoparticle contribute to the cytotoxicity of the tumor cells. The authors note that there were changes to the macrophage, T-cell and NK cell populations and that ancillary immunotherapies should be used in conjunction with the ^{225}Ac nanoparticles

In another study to investigate treatment of glioblastoma[62], liposomes were modified by incorporation of a small-molecule integrin antagonist, that strongly binds to $\alpha_V\beta_3$ integrin that is highly expressed on glioblastoma cells. The $\alpha_V\beta_3$ integrin-specific liposomes modified with DOTA were labeled with actinium at 70 °C for 50 min. These ^{225}Ac labeled $\alpha_V\beta_3$-specific liposomes (^{225}Ac-IA-TLs) enhanced permeability of the blood-brain barrier (BBB) and newly formed tumor vasculature (blood tumor barrier, BTB). Moreover, the resultant complex resulted in double DNA strand breaks within the BBB and the within the BTB.

To test the efficacy of the labeled liposomes athymic nude mice implanted with U87 MG human glioblastoma cells. Ten days post implantation 37 kBq/ 5 μL of the [^{225}Ac]Ac-IA-TLs were injected intravenously. The mice (n= 3) were sacrificed 2, 5, and 10 d post injection to assess the permeability induced by the actinium labeled liposome. Evans Blue dye, which does not permeate the BBB, was injected 6 hours prior to sacrifice to demonstrate the effect of permeability the labeled liposomes had on the BBB. In mice sacrificed 2 days after the injection of the liposomes, "modest" penetration was observed. However, "more extensive" penetration of the dye was observed in mice sacrificed after 5 days Figure 5. The results indicate that ^{225}Ac-IA-TLs increased the potential for permeability across the BBB, and thus are a potential candidate for delivery of therapeutics across the BBB.

Clinical Experiences with ^{225}Ac

^{225}Ac-PMSA-617: The most extensive clinical experience to date has been gained with more than 300 prostate cancer patients with ^{225}Ac-PMSA-617. PMSA-617 is a low molecular weight glu-urea-class of molecules that possesses high affinity to prostate-specific membrane antigen, glutamate carboxypeptidase II (PMSA), an enzyme overexpressed in most prostate tumors. The first study of the treatment of metastatic prostate cancer using [^{225}Ac]AcPSMA-617 in humans was conducted on patients where β therapy ([^{177}Lu]Lu-PMSA-617) was not effective [14]. Two metastatic castrate-resistant-prostate cancer patients presented with late stage disease and widespread metastases and received [^{225}Ac]Ac-PMSA-617 (100kBq/kg, bimonthly) as salvage therapy after the presence of PSMA-positive tumor phenotype had been validated by ^{68}Ga-PMSA-11 PET/CT. After three doses of treatment, both patients showed a complete response, i.e. a dramatic decrease in the presence of the tumors and the concentration of prostate-specific antigen (PSA) in the patient's blood[14]. One of the serious issues with [^{225}Ac]Ac -PMSA-617 is xerostomia, that is, salivary gland toxicity.

Since that report, there have been various clinical experiences of [^{225}Ac]Ac-PMSA- 617 with different treatment regimens. These are summarized in detail in reference[63]. Briefly, three approaches were explored; one approach (dynamic de-escalation) utilized a fixed dose of 8 MBq in the first cycle. At a certain point, the treatment activity is reduced by 2 MBq in the subsequent treatment cycle or increased in the case of prostate specific antigen (PSA) nonresponse. In a second approach, treatment activity was reduced to 6 MBq at the beginning. In a third regimen, cocktails of 4 MBq [^{225}Ac]Ac-PMSA-617 and 4 MBq [^{177}Lu]LuPMSA- 617 were administered, effectively reducing the dose to approximately 50% of the fist approach. Dynamic de-escalation and the cocktail approach improved tolerability without losing too much antitumor activity. Other studies employing the dynamic de-escalation and variations of the cocktail approach where [^{177}Lu]Lu-PMSA-617 were co-administered with [^{225}Ac]Ac- PMSA-617 were discussed[63] and reported to reduce salivary gland toxicity.

^{225}Ac-J591: NCT03276572: This ongoing clinical trial is for men with advanced prostate cancer[64]. The purpose is to find the highest dose level of ^{225}Ac-J591 that can be administered without severe side effects. This phase I Dose-Escalation Trial of ^{225}Ac-J591 in patients with metastatic castration-resistant prostate cancer is estimated to enroll 42 patients. J591 is a monoclonal antibody that recognizes PSMA. The treatment phase is comprised of eight visits over approximately 12 weeks. A single dose will be given on treatment day and subjects will subsequently undergo PET/CT using [^{68}Ga]Ga-PSMA-HBED to document treatment response.

^{225}Ac-Lintuzumab: Lintuzumab is a humanized monoclonal antibody, HuM195, that targets the cell surface antigen CD33 that is expressed on the vast majority of acute myeloid leukemia (AML) cells. [^{225}Ac]Ac-lintuzumab clinical trials was discussed in detail in reference [65]. An initial phase I dose-escalation trial demonstrated that for a single infusion of [^{225}Ac]Ac-lintuzumab in patients with relapsed or refractory acute myeloid leukemia, the maximum tolerated dose (MTD) was determined to be 111 kBq/kg with antileukemic activity across all dose levels. No evidence of radiation-induced nephrotoxicity was seen. Peripheral blasts were eliminated in 63% of the patients at doses of > 37 kBq/kg. Bone marrow blast reduction was observed in 67% of patients [66]. Subsequently, a multicenter phase I dose -escalation trial was conducted to define MTD, toxicity, and response rate of fractionated-dose [^{225}Ac]Ac-lintuzumab when combined with low -dose cytarabine (LDAC) in older patients with untreated AML who were not candidates for intensive chemotherapy [67]. Myelosuppression and infectious complications were common adverse events, and the MTD was not reached as responses were seen with patients receiving > 37 kBq/kg. A phase II trial of [^{225}Ac]Ac-lintuzumab was conducted in patients age 60 years and older [68]. This study resulted in a lower rate of myelosuppression for 22% of patients receiving two 55.5 kBq/kg fractions but a lower response rate than seen for 69% of patients receiving two fractions of 74 kBq/kg.

Patients receiving [^{225}Ac]Ac-HuM195 have also been administered furosemide and spironolactone to prevent potential radiation-induced renal toxicity [69].

^{225}Ac-DOTATOC for therapy of Neuroendocrine tumors: [^{225}Ac]Ac-DOTATOC was investigated in vitro and in preclinical animal models [70]. Therapy using [^{225}Ac]Ac-DOTATOC was clinically tested with 39 patients with progressive neuroendocrine tumors[71] the MTD of a single-cycle [^{225}Ac]Ac-DOTATOC was considered to be 40 MBq while multiple fractions are tolerated with 25 MBq every 4 months or 18.5 MBq every two months.

^{225}Ac substance P analogs for Glioma therapy: ^{225}Ac labeled DOTA-[Thi8, Met (O2)11]-substance P showed promising anti-tumor efficacy[72]. Clinical testing of intratumoral/intercavitary injection of ^{225}Ac labeled DOTA-[Thi8, Met (O2)11]- substance P has been started at Medical University Warsaw in collaboration with JRC Karlsruhe. To date, >20 glioma patients have been treated with activities ranging from 10 to 42 MBq ^{225}Ac labeled DOTA-[Thi8, Met (O2)11]- substance P. The treatment has been well tolerated, analysis of therapeutic efficacy, dose escalation and patient recruitment is ongoing [73].

Thorium-227

Chemical Properties and Decay—Thorium is an oxophilic metal with complicated coordination chemistry. At high pH (>7), thorium precipitates out of solution in the form of various water insoluble oxides thus making coordination difficult. Thorium is commonly found in the +4-oxidation state. The standard reduction potential of the Th(IV)/Th(III) couple is −3.7V[74] which makes reduction difficult and makes the metal generally redox inactive. While thorium is a +4 metal, it can have coordination numbers from 4–15, the most common coordination number being eight[75]. Thorium-227 has a half-life of 18.7 days and decays via alpha particle emission to radium-223 ($t_{1/2}$= 11.4 d), Radon-219 ($t_{1/2}$= 3.96 s), polonium-215 ($t_{1/2}$=1.78 ms), lead-211 ($t_{1/2}$=36.1 min) , bismuth-211 ($t_{1/2}$= 2.1 min), thallium-207 ($t_{1/2}$= 4.77 min), and finally lead- 207 (Stable). In less than one half-life, ^{227}Th and ^{223}Ra exist in transient equilibrium. Two of the daughters of radium-223 decay are lead isotopes, ^{211}Pb and ^{207}Pb, which can compete with thorium for ligands in a labeling reaction. Both lead and thorium can exist as +4 metals and can be coordinated to the same ligand.

Production—Thorium-227 is obtained from the decay of ^{227}Ac ($t_{½}$= 21.8 years). The main source of ^{227}Ac is neutron irradiation of ^{226}Ra with thermal neutrons to produce ^{227}Ra which decays via beta emission to ^{227}Ac. The neutron irradiation can produce ^{227}Ac in GBq quantities[76] and be efficiently separated. The ^{227}Th obtained from ^{227}Ac, also contains ^{223}Ra which is separated following the ^{227}Th elution using ion chromatography resins. To perform this separation Boll *et. al.*[30] used the MP1 anionic exchange resin, while McAlister *et. al.*[77] used a combination of resins (UTEVA, DGA) and a prefilter resin.

To achieve optimal radiolabeling and subsequent analysis ^{227}Th must be chelated immediately upon receipt or purified prior to radiolabeling. This eliminates interference with the daughters and ensures that only ^{227}Th is available for labeling. Labeling with partially decayed thorium reduces the radiolabeling yields of ^{227}Th, and more importantly, due to the unlabeled daughter isotopes, may increase the toxicity *in vivo*. ^{227}Th can be purified using an anion exchange column. Thorium forms the [^{227}Th][$Th(NO_3)_6$]$^{2-}$ complex at high

concentrations of nitric acid (>7 M) [18, 30, 34, 78–80], and binds strongly to anion exchange resins. The daughters of thorium (radium, radon, polonium, lead, bismuth, thallium), do not form these anionic complexes in high concentrations of nitric acid, and elute from the column in the loading fraction as free metals. Thorium can be eluted by lowering the acid concentration and decomposing the $[^{227}Th][Th(NO_3)_6]^{2-}$ complex. The employment of an anion exchange resin to purify or separate thorium is a convenient and important approach to ensure the purity of thorium.

The first decay daughter of ^{227}Th is ^{223}Ra, Figure 6, which will not radiolabel with conventional ligands, however, ^{223}Ra contributes as a radioactive impurity that can lead to toxicity through bone localization. One of the daughters of ^{223}Ra, ^{211}Pb ($t_{1/2}$= 36 minutes, β- 1.38 MeV), can compete with thorium for labeling with conventionally used chelators, especially DOTA. Lead can exist in either the +2 or +4 oxidation state. The ligand DOTA binds very well to Pb^{+4} (log ß = 24.3)[81] and this will reduce yields of the Th^{+4} DOTA product. The presence of ^{211}Pb can be a problem as it will compete for uptake with ^{227}Th and therefore, reduce the amount of ^{227}Th going to the target site. The other daughters of ^{223}Ra will not bind DOTA efficiently and can redistribute and contribute to toxicity *in vivo*. One way to circumvent the presence of the ^{223}Ra daughters in radioimmunotherapy (RIT) is to conduct experiments with thorium labeled antibodies using freshly purified thorium or perform a separation to isolate the complexed thorium[82].

Thorium Labeling and Preclinical Studies—For all preclinical studies ^{227}Th was eluted from a ^{227}Ac generator in 12 M HCl, evaporated to dryness and the residue was suspended or dissolved in 0.01M HCl or 0.1M HNO_3 and buffered for complexation. In early studies by Dahle and co-workers[83], ^{227}Th was originally conjugated to the antibody, rituximab, an anti-CD20 monoclonal antibody. This work used the bifunctional chelator, p-SCN-Bn-DOTA to coordinate ^{227}Th to the antibody. ^{227}Th eluted from the ^{227}Ac generator was evaporated to dryness and the residue resuspended in 0.01M HCl. Subsequently the ^{227}Th suspension was added to p-SCN-Bn-DOTA buffered at pH 5.5 for 40 min at 55 – 60ºC. The $[^{227}Th]$Th-p-SCN-Bn-DOTA complex was then cooled to 37ºC and reacted with rituximab at pH 8–9 for 45 min. The thorium labeled antibodies demonstrated *in vitro* and *in vivo* stability with ≥75% of the labeled antibody remaining intact after 7 days[84]. The overall radiolabeling yield was 17% and the immunoreactivity of the $[^{227}Th]$Th-DOTA-rituximab was 56–65%. The specific activity 500–1000 kBq/ug. The challenge with this work is that the authors do not say how many DOTA per antibody which may explain the low immunoreactivity.

$[^{227}Th]$Th-DOTA-rituximab (200–1000 kBq/kg) was administered to mice bearing Raji lymphoma xenografts resulting in delayed tumor growth and prolonged mean survival when compared with a control group which were injected with cold rituximab. The mice did not experience serious toxicity. A significant tumor growth delay was achieved with a dose of 1000 kBq /kg (40 days) compared to the dose of 200 kBq /kg (17 days). The ^{227}Th low-dose strategy appeared to be effective in the treatment of macroscopic tumor and single tumor cells[85]. A study was completed comparing the relative biological effects of low-dose and low dose-rate alpha radiation from $[^{227}Th]$Th-rituximab to Zevalin ($[^{90}Y]$Y-antibody) and external beam radiation. It was demonstrated that treatment with ^{227}Th was 2.5 to

7.2 times more effective in inhibiting tumor growth than external beam radiation. Beta radiation treatment with [^{90}Y]Y-Zevalin was 1 to 1.3 times more effective than external beam radiation[86]. Nasir and coworkers[87] also compared the therapeutic effects of ^{177}Lu and ^{227}Th on HER2 expressing ovarian cancer tumors. Here, ^{227}Th demonstrated a greater antitumor effect than ^{177}Lu using the 4 Gy of radiation. The tumor growth was delayed further with ^{227}Th and the mean survival of the mice was longer (129 days vs 88 days) Figure 7.

Investigation of [^{227}Th]Th-rituximab for long-term radiotoxicity involved injection of the labeled construct into tumor bearing animals at 52, 200, and 1000 kBq /kg and observation for one year. A total of 120 mice treated with 1000 kBq /kg experienced weight loss and a decrease in white blood cells and platelet count. This study determined that the "no-observed-adverse-effect" level was 200 kBq/kg and the "maximum tolerated activity" was determined to be between 592 –1000 kBq/kg.[88] In these studies, [^{227}Th]Th-DOTA-trastuzumab was evaluated in two HER-2 positive breast cancer cell lines (BT-474 and SKBR-3) and in the ovarian cell line SKOV-3. [^{227}Th]Th-DOTA-trastuzumab inhibited cell growth and induced apoptosis in all cell lines at doses that were clinically relevant[89]. In fact, doses as low as 51.8 kBq/kg of ^{227}Th antibody resulted in complete inhibition of tumor growth in mice bearing human renal cell carcinoma subcutaneous xenografts[90]. Myelosuppression was observed in days 44–65. However, the mice recovered by day 114 and the toxicity was considered transient. The recovery time is consistent with previously reported literature[91].

DOTA is the ligand typically used for binding ^{227}Th as it is FDA approved and commercially available. DOTA–antibody labeling has also been reported for ^{227}Th by a 2-step method[83] and by a 1-step method wherein the labeling proceeded overnight to afford high yield of the [^{227}Th]Th-DOTA-antibody[92]. Both of these methods are not optimal. The "2-step" method used an isothiocyanate C-functionalized derivative of the DOTA chelator first radiolabeled with ^{227}Th and then conjugated to the antibody at 37˚C.

Ramdahl prepared Me-2,3-HOPO, that is based on the methyl-2,3-hydroxypyridinone and is an 8-coordinate ligand[93] as well as a bifunctional version of Me-2,3-HOPO Figure 8. The bifunctional Me-2,3-HOPO was conjugated to the CD33-targeting antibody lintuzumab and radiolabeled with ^{227}Th. The ^{227}Th construct induced *in vitro* cytotoxicity on CD33-positive cells, manifested by accumulated DNA double-strand breaks after exposure to the [^{227}Th]Th-lintuzumab. In a subcutaneous xenograft mouse model using HL-60 cells, the ^{227}Th conjugate demonstrated antitumor activity in a single dose injection of 500 kBq/kg. Dose-dependent survival was demonstrated in a mouse tumor model after single dose injection or after fractionated dose administration[91].

Preclinical investigation of ^{227}Th labeled PSMA-IgG antibody provides encouragement for future clinical trials with this construct. ^{227}Th-PMSA-IgG demonstrated tolerability and antitumor activity in several prostate cancer xenograft models. Tumor growth inhibition was observed with a single dose of ^{227}Th-PMSA-IgG in prostate cancer xenograft models with moderate and high PMSA expression. In bone-metastatic PC model, a single dose of ^{227}Th-PMSA-IgG reduced tumor burden, abnormal osteoblastic growth and serum PSA

levels[94]. Dose -dependent antitumor activity was observed in hormone-sensitive patient derived PC xenograft models[95].

Clinical Trials—Bayer is conducting clinical trials with ^{227}Th however, the results of these trials have not been published as the trials are ongoing or have only recently ended. Currently, there are three clinical trials which are actively recruiting participants. The first, BAY 2315497, is a study to evaluate the safety, tolerability, pharmacokinetics and anti-tumor activity of a thorium-227 labeled antibody-chelator conjugate in patients with metastatic castration resistant prostate cancer[96]. Another study, BAY2701439 will be recruiting patients[96]. This is a first in human study to examine safety, how well the drug works in patients with advanced cancer where the protein HER2 is expressed. The study will include patients with HER2 expressing breast, gastric, or gastroesophageal cancers and any cancers that express HER2. The objective is to find the best dose for patients and to understand the pharmacokinetics of the drug. Another clinical study in the recruiting phase is the first human injection of BAY2287411, a thorium-227 labeled antibody-chelator conjugate, in patients with tumors that express mesothelin[96]. The purpose is to evaluate the safety, tolerability, maximum tolerated dose, anti-tumor activity, pharmacokinetics and recommended dose for further clinical development[97].

Bayer has completed one study of the safety and tolerability of BAY1862864 injection in subjects with relapsed or refractory CD-2 positive Non-Hodgkins lymphoma[98]. This study concluded in December of 2019 and the results have not been reported at the time of this publication.

Conclusions

Recent approval of NetSpot and Lutathera has heightened interest in the use of targeted radiotherapy particularly to treat patients with metastatic disease. The first radionuclides conjugated to targeting molecules and used to treat disseminated disease have been used with the most studied beta emitting radionuclides, iodine-131 and yttrium-90. Largely due to the toxicity observed for Y-90, investigators began evaluating lutetium-177 as it could be imaged and its lower beta range resulted in lower toxicity to the kidney and normal tissues. Further it could be made in quantities sufficient to support clinical trials and drug development. However, although the use of ^{177}Lu in clinical trials is safe it is not always effective in sterilizing disseminated disease. The shorter particle range and high LET of alpha particles offer the ability to deliver significant dose to tumor cells while reducing toxicity. A major stumbling block for using alpha emitting radionuclides was their limited availability and the need for improved chelators or labeling methods to ensure they remain stably complexed in vivo and deliver their payload to the tumor sites and minimize the dose that can arise due to dissociation. New production methods have come online such as the production of ^{225}Ac via the high energy accelerator irradiation of ^{232}Th which can result in 10s to 100 Curie levels of production. The DOE isotope program is now routinely supplying ^{225}Ac produced in this manner and has demonstrated it to be similar to ^{225}Ac obtained from the ^{229}Th generator. This means supply is now at a level to support pre-clinical as well as clinical studies on a routine basis. Recently, the DOE submitted a drug master file to the FDA to support its clinical use. Further, other routes of production are being pursued

such as the photonuclear route on ^{226}Ra and the low energy cyclotron route on ^{226}Ra. Thorium-227 which was not even available for pre-clinical studies is now available from multiple sites including DOE as a byproduct from ^{227}Ac. This means supply is now at a level to support pre-clinical as well as clinical studies on a routine basis. New ligands are also being developed for both isotopes such as macropa, HEHA, and Me-2,3- HOPO which offer enhanced stability and mitigate toxicity. Additionally, some of the new liposomal or nanoparticle delivery systems could offer the ability to deliver higher doses and mitigate release of daughters to neighboring tissues. A further enhancement is the development of dosimetry methods for alpha particle emitting radionuclides that can further facilitate personalized medicine. We appear to be entering a phase where many advancements are finally allowing for the promise of targeted alpha therapy to come into existence. Although many see this as a new area, it is actually an area that was under investigation for some time and researchers are strongly recommended to go back and look at the pioneering work that was done. Due to technological advancements in targetry, production, chelation, detection, and dosimetry we can now build on this earlier work to facilitate the use of ^{225}Ac and ^{227}Th in both the pre-clinical and clinical setting.

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98. Safety and Tolerability of BAY1862864 Injection in Subjects With Relapsed or Refractory CD22-positive Non-Hodgkin's Lymphomahttps://clinicaltrials.gov/ct2/show/NCT02581878?term=thorium&cond=Non+Hodgkin+Lymphoma&draw=2&rank=1).

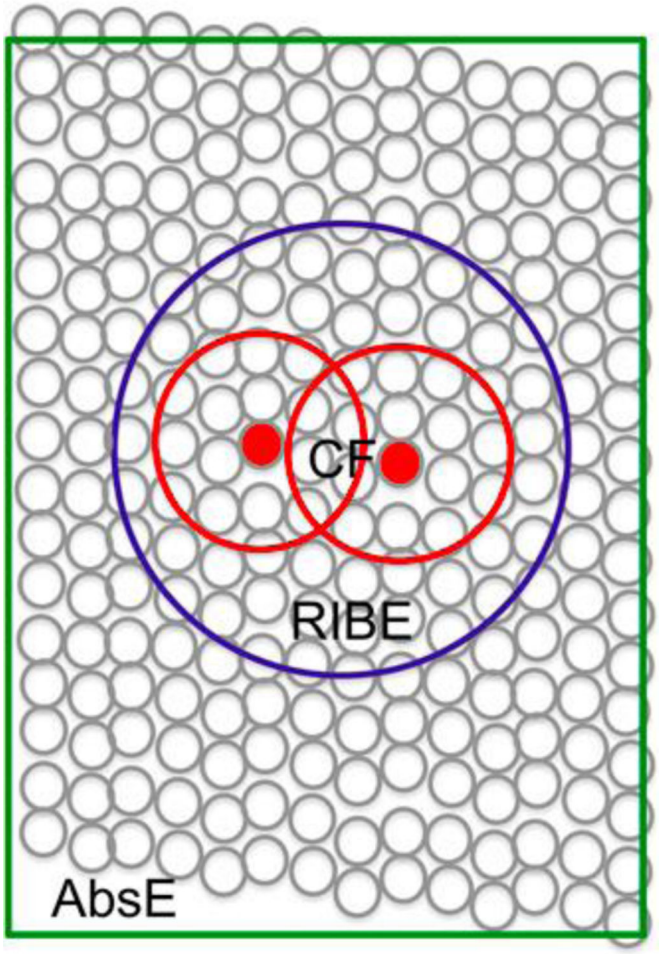

Figure 1.
schematic describing the crossfire (CF) effect, radiation-induced bystander (RIBE) effect, and the abscopal (AbsE) effect (see text) this image was originally published in JNM. Haberkorn,U., Giesel, F., Morgenstern, A., Kratochwil, C The Future of Radioligand Therapy: alpha, beta or both? J Nucl Med. 2017;7:1017–1018. © SNMMI [9]

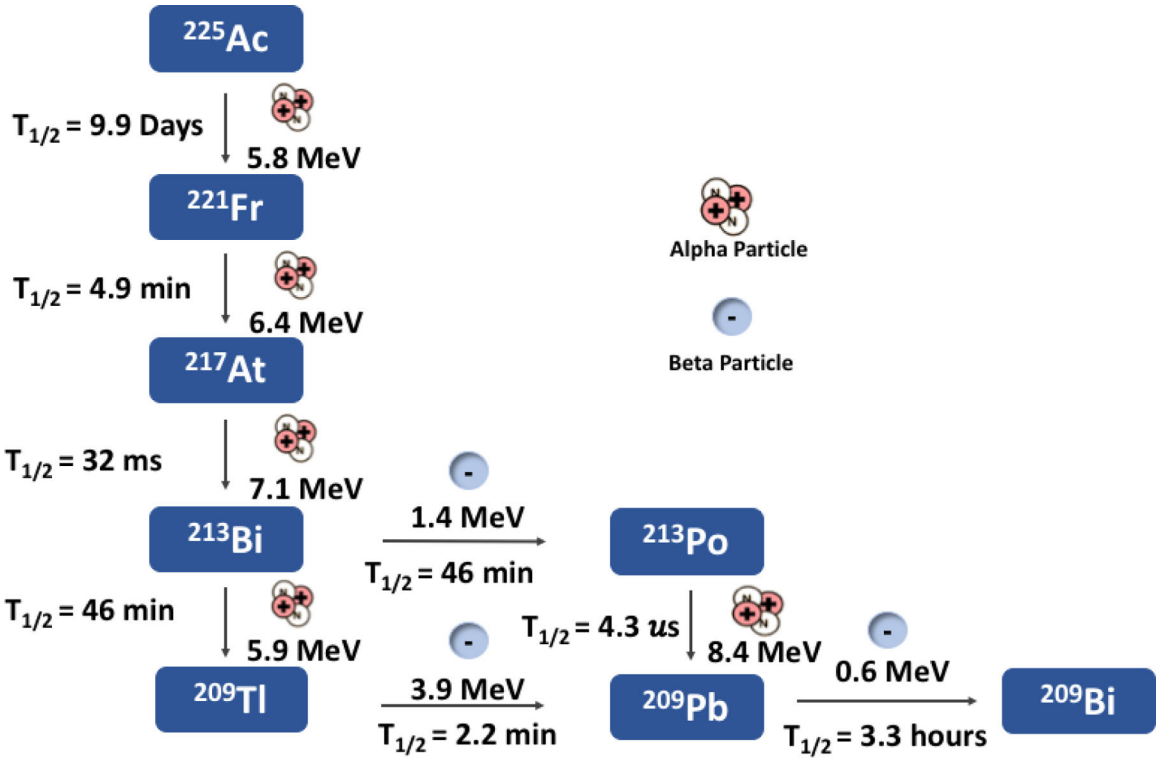

Figure 2.
Ac-225 decay chain

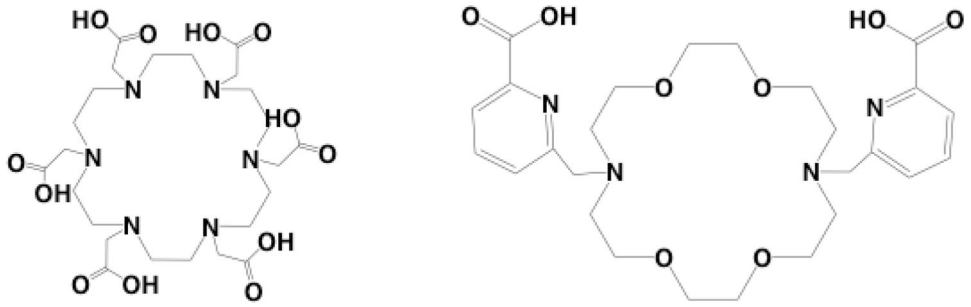

Figure 3.
Structures of HEHA(left) and Macropa (Right)

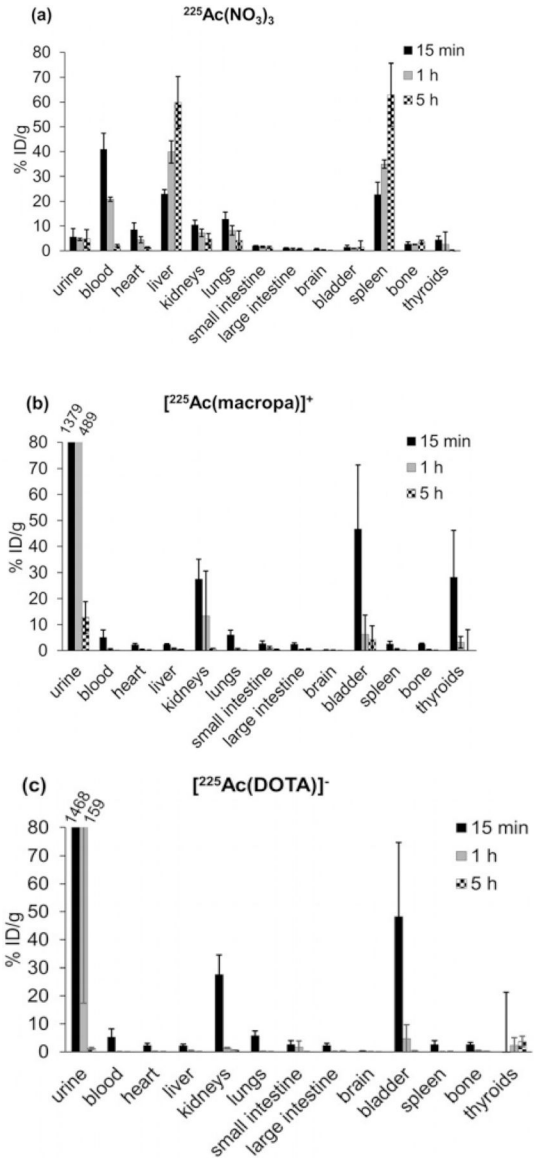

Figure 4 (a–c).
Biodistributions of Ac[NO_3], Ac[Macropa], and Ac[DOTA]

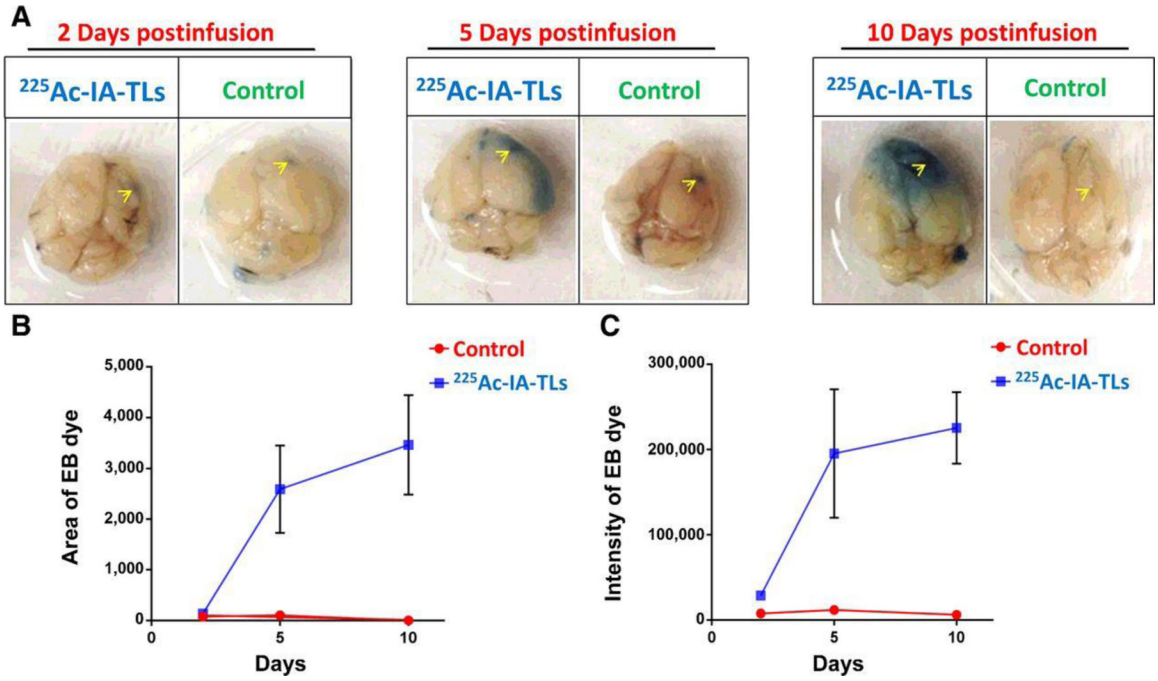

Figure 5.

^{225}Ac-IA-TLs result in BBB opening in immunocompetent mice. A. BBB opening in immunocompetent mice infused with ^{225}Ac-IA-TLs 2 days, 5 days and 10 days post injection compared to saline infused immunocompetent mice as demonstrated by intraveneously injected Evans Blue dye. Site of intracranial injection marked by yellow arrows. B and C: Area and intensity of Evans Blue dye was found to be greater in mice that were intracranially infused with ^{225}Ac-IA-TLs when compared with the saline control up to 10 days postinfusion[62].

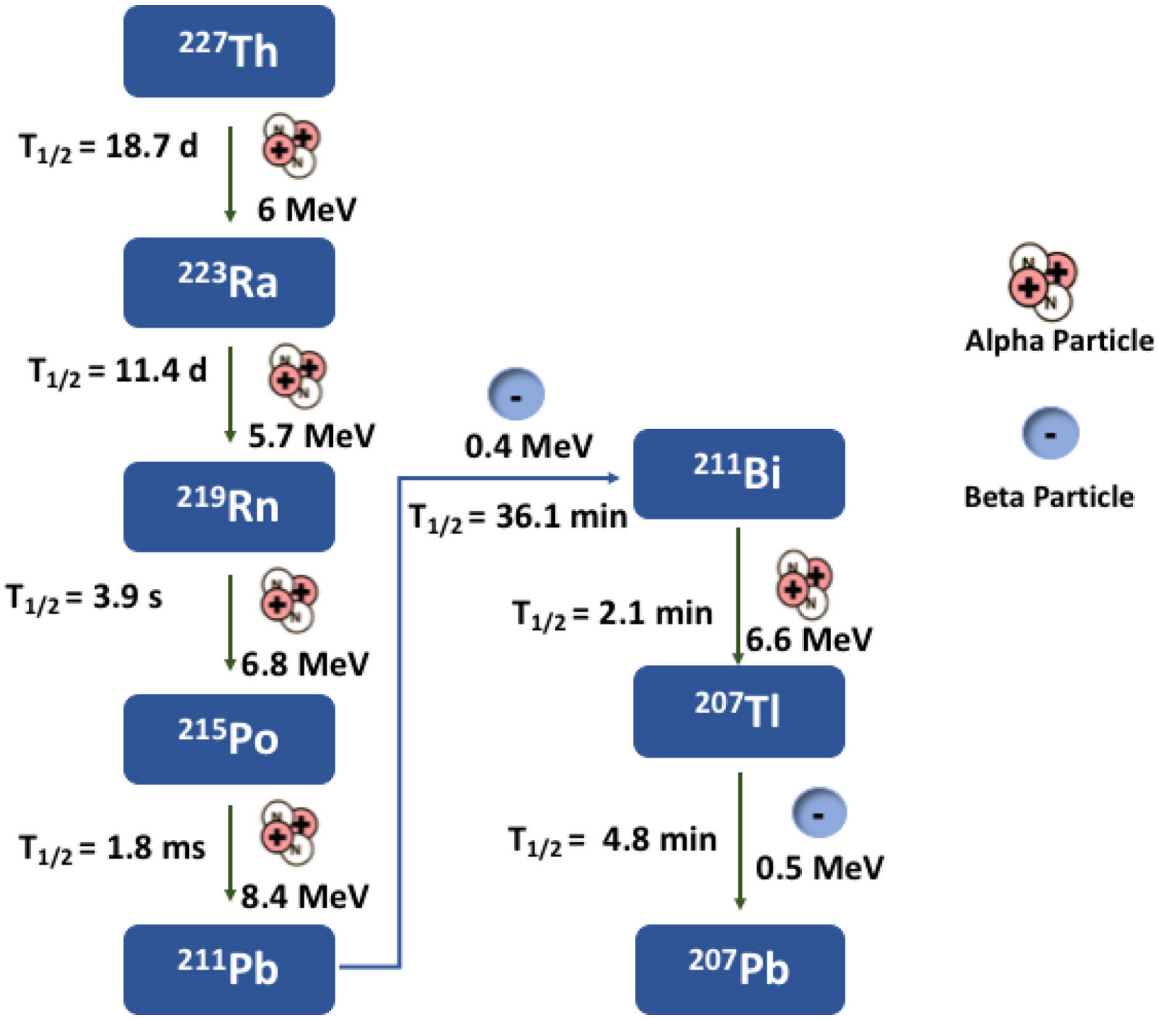

Figure 6.
Decay Scheme of Th-227.

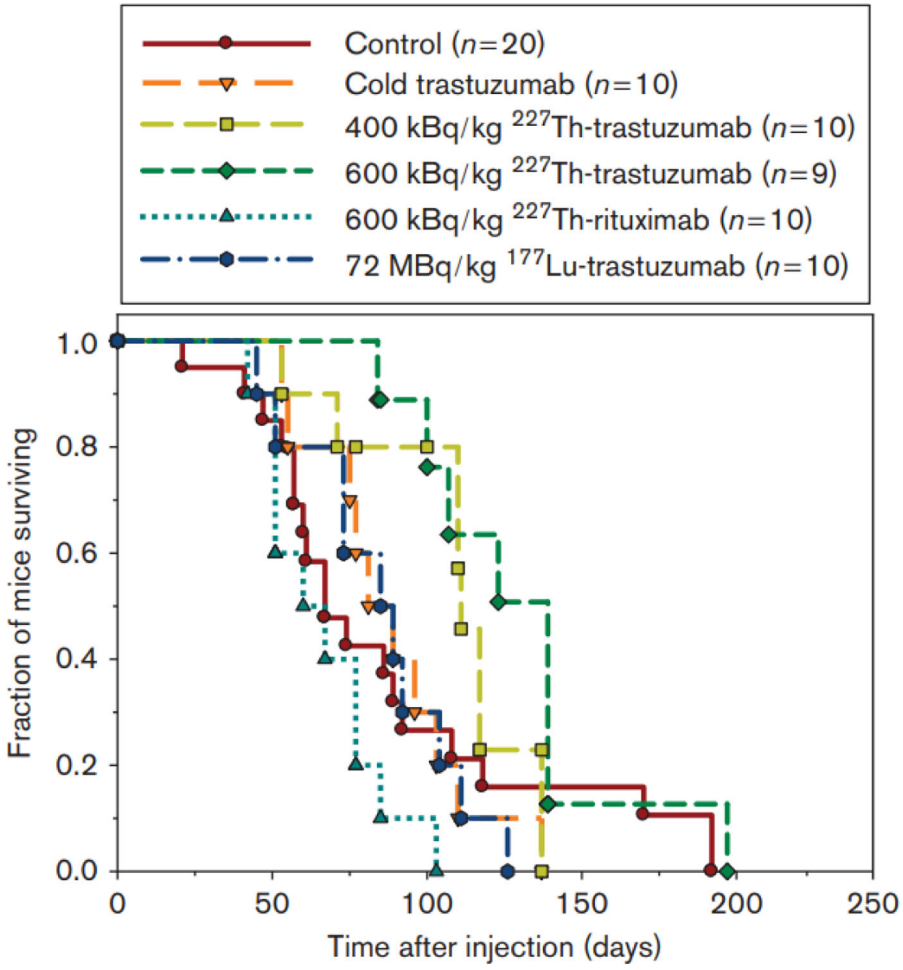

Figure 7.

The Kaplan-Meier survival plot of mice treated with ^{177}Lu-trastuzumab and ^{227}Th-Trastuzumab.

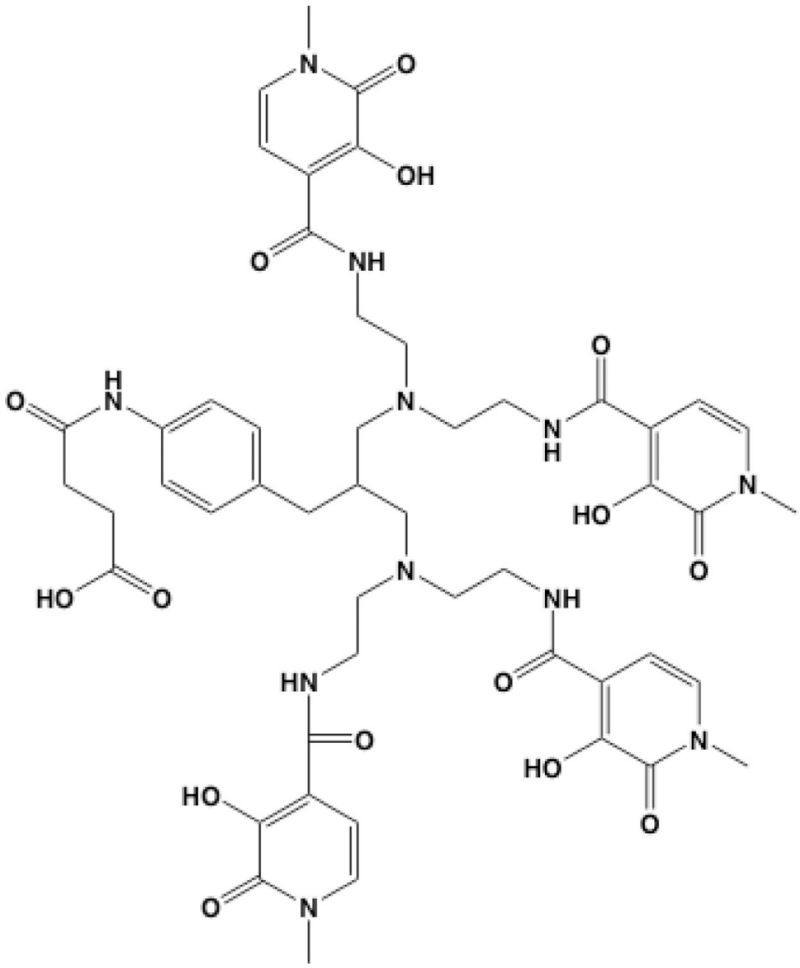

Figure 8.
Me-2,3-HOPO